BIOSPECTRA
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GLYCERIN TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory Personnel with procedures for testing Glycerin.

2. SCOPE:

2.1. Applies to the testing of Glycerin in the laboratory. Methods include testing for all types of Glycerin sold by BioSpectra; only the specific tests required for the desired type must be tested.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager is responsible for the control, training, maintenance and implementation of this procedure.
3.2. The Laboratory Technicians and Analytical Chemistry Specialists are responsible for compliance with the terms of this procedure. This includes notifying the Quality Assurance / Laboratory Managers or designee if any analyses fail to meet their respective specifications.
3.3. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCES:

4.1. BSI-ATM-0050, Analytical Method: Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine by GC MC
4.2. BSI-ATM-0134, Limit of Related Substances in Glycerin via GC-FID
4.3. BSI-ATM-0135, Limit of Specified Impurities in Glycerin by HPLC
4.4. BSI-ATM-0136, Residual Solvents by Headspace GC-FID: Glycerin
4.5. BSI-PRL-0348, Analytical Method Validation Protocol: Aqueous Soluble Residual Solvents USP 1467
4.6. BSI-PRL-0740, Analytical Method Verification Protocol: Glycerin Water Determination Via Karl Fischer Utilizing Metrohm 907 Auto-Titrator
4.7. BSI-PRL-0743, Analytical Method Validation Protocol: Limit of Related Substances in Glycerol by GC-FID
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4.9. BSI-PRL-0791, Analytical Method Validation Protocol: Glycerin Assay by Potentiometric Titration with 0.1N Sodium Hydroxide
4.10. BSI-PRL-0866, Analytical Method Validation Protocol: Glycerin Specified Impurities by Derivatization with O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride (PFBHA) Via Liquid Chromatography with UV Detection
4.11. BSI-RPT-1639, Analytical Method Validation Report: Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin
4.12. BSI-RPT-1813, Analytical Method Verification Report: Glycerin Water Determination via Karl Fischer Utilizing Metrohm 907 Auto-Titrator
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4.15. BSI-RPT-2187, Analytical Method Verification Report: Quantification of Formaldehyde in Glycerin by Derivatization with Pentafluorobenzylhydroxyl Amine
4.16. BSI-RPT-2196, Analytical Method Validation Report: Residual Solvents by Head Space GC-FID – Glycerin
4.17. BSI-RPT-2197, Analytical Method Validation Report: Glycerin Specified Impurities by Derivatization with O-(2,3,4,5,6-Pentafluorobenzyl) Hydroxylamine Hydrochloride (PFBHA) Via Liquid Chromatography with UV Detection

4.18. BSI-SOP-0090, Lambda 25 UV/VIS Operation and Calibration
4.19. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
4.20. BSI-SOP-0094, Muffle Furnace SOP and Calibration
4.21. BSI-SOP-0098, Balance SOP
4.22. BSI-SOP-0126, Laboratory Notebooks
4.23. BSI-SOP-0134, Pipette SOP
4.24. BSI-SOP-0135, Laboratory Chemicals
4.25. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.26. BSI-SOP-0161, Waste Handling SOP
4.27. BSI-SOP-0254, Spectrum Two UATR SOP
4.28. BSI-SOP-0259, Fisher Scientific Isotemp Water Bath Operation Calibration SOP
4.29. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.30. BSI-SOP-0308, Pycnometer SOP
4.31. BSI-SOP-0316, Shimadzu QP2010S GC/MS SOP
4.32. BSI-SOP-0342, Fisherbrand™ Handheld Digital Brix/RI Refractometer
4.33. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
4.34. BSI-SOP-0473, Rainin Pipette SOP
4.35. *Chinese Pharmacopoeia, Current Edition*
4.36. *European Pharmacopoeia, Current Edition*
4.37. *Japanese Pharmacopoeia, Current Edition*
4.38. *United States Pharmacopoeia, Current Edition*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Auto-Titrator
5.3. Beakers, Various Sizes
5.4. Bunsen Burner
5.5. Burette
5.6. Calibrated Micropipettes
5.7. Calibrated Timers
5.8. Calibrated Turbidimeter
5.9. Class A Volumetric Flasks
5.10. Digital Refractometer
5.11. Endosafe NexGen PTS Endotoxin Reader
5.12. Gas Chromatograph with Flame Ionization Detection (GC-FID)
5.13. Gas Chromatograph with Mass Spectrometer (GC-MS)
5.14. High Performance Liquid Chromatograph (HPLC) with UV Detection
5.15. Hot Plates
5.16. Inductively Coupled Plasma Mass Spectrometer (ICP-MS)
5.17. Infrared Spectrometer with UATR
5.18. Milli-Q IQ 7005 with IQ-Element and Q-Pod Water Purification System
5.19. Muffle Furnace
5.20. Nessler Color Comparison Tubes
5.21. Calibrated Oven
5.22. pH/mV/Conductivity Meter
5.23. pH Paper
5.24. pH Probe
5.25. Pycnometer
5.26. Reflux Condenser
5.27. Round-Bottom Flasks
5.28. Stir Bars

5.29. Stir Plate
5.30. Transfer Pipettes
5.31. UV/Vis Spectrophotometer
5.32. Water Bath

6. REAGENTS:

6.1. **0.02N Hydrochloric Acid:** Purchased Commercially.
6.2. **0.02N Sodium Hydroxide:** Dilute 2mL of 1N Sodium Hydroxide to 100mL with Purified Water. Scale as needed.
6.3. **0.02N Sulfuric Acid:** Dilute 20mL of 0.1N sulfuric acid to 100mL with purified water. Scale as needed.
6.4. **0.01N Sulfuric Acid:** Dilute 10mL of 0.1N sulfuric acid to 100mL with purified water. Scale as needed.
6.5. **0.05N Sodium Hydroxide:** Dilute 5mL of 1N Sodium Hydroxide to 100mL with purified water and mix well.
6.6. **0.1N Hydrochloric Acid:** Purchased Commercially.
6.7. **0.1N Silver Nitrate:** Purchased Commercially.
6.8. **0.1N Sodium Hydroxide:** Purchased Commercially.
6.9. **0.1N Sodium Thiosulfate:** Purchased Commercially.
6.10. **0.1N Sulfuric Acid:** Purchased Commercially.
6.11. **0.2N Sulfuric Acid:** Dilute 20mL of 1N Sulfuric Acid to 100mL with Purified Water and mix well.
6.12. **0.5% Ferric Chloride Solution:** Weigh 0.5g of Ferric Chloride Hexahydrate and dilute to 100mL with Purified Water.
6.13. **0.5N Hydrochloric Acid:** To a 1000mL volumetric flask containing 40mL of Purified Water, slowly add 43mL of concentrated Hydrochloric Acid, cool, fill to volume with Purified Water, and mix thoroughly.
6.14. **0.5N Sodium Hydroxide:** Dilute 50mL of 1N Sodium Hydroxide to 100mL with Purified Water. Scale as needed.
6.15. **1N Hydrochloric Acid:** Purchased Commercially.
6.16. **1N Sodium Hydroxide:** Purchased Commercially.
6.17. **1N Sulfuric Acid:** Purchased Commercially.
6.18. **2N Sodium Hydroxide:** Weigh 20.0 grams of Sodium Hydroxide Pellets, dissolve in Purified Water, allow to cool to room temperature, fill to volume with Purified Water, and mix thoroughly.
6.19. **3-Methyl-2-Benzothiazolinone Hydrazone Hydrochloride Hydrate:** Purchased Commercially.
6.20. **3N Hydrochloric Acid:** Pipette 25.75 mL of concentrated hydrochloric acid and transfer to a 100-mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.
6.21. **10% Methylbenzo-2-Thiazolonehydrazone Hydrochloride Solution:** Weigh 10.0g of 3-Methyl-2-Benzothiazolinone Hydrazone Hydrochloride Hydrate and dilute to 100mL with purified water.
6.22. **10% Potassium Hydroxide:** Weigh 10.0g of Potassium Hydroxide and dilute to 100mL with Purified Water.
6.23. **10% Sodium Hydroxide:** Dilute 20mL of 50% Sodium Hydroxide solution to 100mL with Purified Water.
6.24. **16% (w/v) Formaldehyde Reference Standard:** Purchased Commercially.
6.25. **25% Barium Chloride:** Dissolve 25 grams of Barium Chloride Dihydrate in Purified water, dilute to 100mL with Purified Water, and mix thoroughly.

6.26. **85mg/mL (8.5%) Sodium Hydroxide:** Weigh 8.5g of Sodium Hydroxide Pellets and dilute to 100mL with Purified Water.
6.27. **Ammonia TS:** To 40mL of Ammonium Hydroxide (28%) add Purified Water to make 100mL.
6.28. **Ammonium Hydroxide (28%):** Purchased Commercially.
6.29. **Barium Chloride TS:** Dissolve 30g of Barium Chloride Dihydrate in Purified Water; dilute to 250mL and mix thoroughly.
6.30. **Barium Chloride Dihydrate:** Purchased Commercially.
6.31. **Bromothymol Blue:** Purchased Commercially.
6.32. **Bromothymol Blue TS:** Dissolve 0.1 grams of Bromothymol Blue in 100mL of Reagent Alcohol, and filter if necessary.
6.33. **Chloride Stock Solution (500ppm Cl^-):** Weigh and transfer 0.824 grams of Sodium Chloride to a 1000mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
6.34. **Cobaltous Chloride CS:** Purchased Commercially.
6.35. **Composite 5:** Purchased Commercially.
6.36. **Copper Sulfate:** Purchased Commercially.
6.37. **Copper Sulfate Solution R:** Weigh 12.5g of Copper Sulfate and dilute to 100mL with Purified Water.
6.38. **Decolorized Pararosaniline Solution R:** Purchased Commercially.
6.39. **Dilute Hydrochloric Acid:** Dilute 23.6mL of concentrated Hydrochloric Acid to 100mL with Purified water and mix thoroughly.
6.40. **Dilute Nitric Acid:** Dilute 10.5mL of Concentrated Nitric Acid with Purified Water to make 100mL. Scale as needed.
6.41. **Dilute Nitric Acid R:** Dilute 20g of concentrated Nitric Acid to 100mL with Purified Water.
6.42. **Dilute Sodium Hydroxide Solution R:** Weigh 8.5g of sodium hydroxide and dilute to 100mL with purified water.
6.43. **Dilute Sulfuric Acid R:** Add 5.5mL of concentrated Sulfuric Acid to 60mL of Purified Water. Allow to cool and then dilute to 100mL with Purified Water.
6.44. **Endotoxin Cartridges:** Purchased Commercially.
6.45. **Ethylene Glycol:** Purchased Commercially.
6.46. **Ferric Chloride CS:** Purchased Commercially.
6.47. **Ferric Chloride Hexahydrate:** Purchased Commercially.
6.48. **Formamide Dry:** Purchased Commercially.
6.49. **Glycerin:** In-House or Purchased Commercially.
6.50. **Hydrochloric Acid, concentrated:** Purchased Commercially.
6.51. **LAL Reagent Water:** Purchased Commercially.
6.52. **Litmus Paper:** Purchased Commercially.
6.53. **Matching Fluid H:** Thoroughly mix 0.2mL of Cobaltous Chloride CS, 1.5mL of Ferric Chloride CS, and 3.3mL of Purified Water.
6.54. **Methanol Dry:** Purchased Commercially.
6.55. **Methanol:** Purchased Commercially.
6.56. **Morpholine:** Purchased Commercially.
6.57. **Nickel-Aluminum Alloy:** Purchased Commercially.
6.58. **Nitric Acid, concentrated:** Purchased Commercially.
6.59. **Phenolphthalein Solution R**: Dissolve 0.1g of Phenolphthalein in 80mL of Reagent Alcohol and dilute to 100mL using purified water.
6.60. **Phenolphthalein TS:** Dissolve 1.0g of Phenolphthalein in 100mL of Reagent Alcohol.
6.61. **Phenolphthalein:** Purchased Commercially.
6.62. **Potassium Dichromate:** Purchased Commercially.

6.63. **Potassium Dichromate CS:** Weigh and transfer 0.400 grams of Potassium Dichromate, previously dried to a constant weight at 120°C, to a 500mL volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix thoroughly or Purchased Commercially.
6.64. **Potassium Hydrogen Sulfate:** Purchased Commercially.
6.65. **Potassium Hydroxide:** Purchased Commercially.
6.66. **Potassium Iodide TS:** Dissolve 16.5 grams of Potassium Iodide in Purified Water, dilute to 100mL with Purified Water, and mix well. Store in a light-resistant container.
6.67. **Potassium Iodide:** Purchased Commercially.
6.68. **Potassium Sulfate:** Purchased Commercially.
6.69. **Potassium Sulfate Standard Solution:** Dissolve 0.181g of Potassium Sulfate in Purified Water in a 1000mL volumetric flask. Dilute to volume with Purified Water and mix well (each mL of *Potassium Sulfate Standard Solution* is equivalent to 100µg of sulfate [SO_4]).
6.70. **Previously Dried Potassium Hydrogen Phthalate:** Prepare an appropriate sample container at 120°C for 30 minutes. Allow to cool in a desiccator. Crush and dry a suitable amount of Potassium Hydrogen Phthalate. Dry at 120°C for 2 hours. Cool and store in a desiccator in a closed container. Stable for 3 months.
6.71. **Previously Dried Tromethamine:** Prepare an appropriate sample container at 105°C for 30 minutes. Allow to cool in a desiccator and weigh an appropriate amount of Tris. Dry at 105°C for 3 hours. Cool and store in a desiccator in a closed container. Stable for 3 months.
6.72. **Purified Water:** In-House or Purchased Commercially.
6.73. **Reagent Alcohol:** Purchased Commercially.
6.74. **Silver Nitrate Solution R2:** Use 0.1N Silver Nitrate Solution.
6.75. **Sodium Chloride Standard Solution:** Dissolve 0.165g of Sodium Chloride in Purified Water in a 1000mL volumetric flask. Dilute to volume with Purified Water and mix well (Stock Solution). Immediately before use, transfer 10mL of the *Stock Solution*, accurately measured, into a 100mL volumetric flask. Dilute to volume with Purified Water and mix well (each mL of *Sodium Chloride Standard Solution* is equivalent to 10µg of chlorine).
6.76. **Sodium Chloride:** Purchased Commercially.
6.77. **Sodium Hydroxide Pellets:** Purchased Commercially.
6.78. **Sodium Metaperiodate:** Purchased Commercially.
6.79. **Starch TS:** Purchased Commercially.
6.80. **Sulfuric Acid, Concentrated:** Purchased Commercially.

7. ANALYTICAL PROCEDURES:

Note: All solutions may be scaled as needed.

7.1. SOLUTION S:

7.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well.

7.2. ACIDITY OR ALKALINITY (EP) (ChP):

7.2.1. **Note:** Retain the final solution for Esters (EP) analysis.

7.2.2. Sample Preparation:

7.2.2.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well (Solution S may be utilized).

7.2.3. Analysis:

7.2.3.1. To 50mL of Sample Preparation, add 0.5mL of Phenolphthalein Solution R.

7.2.3.2. The solution should be colorless.

7.2.3.3. Add 0.2mL of 0.1N Sodium Hydroxide, the solution should turn pink to report as passes test.

7.3. ACIDITY OR ALKALINITY (JP):

7.3.1. To 2mL of sample, add 8mL of Purified Water, and mix well.

7.3.2. The solution is neutral (between pH 6-8) when tested with pH Paper to report as Passes Test.

7.4. ALDEHYDES (EP):

7.4.1. Sample Preparation:

7.4.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well. (Solution S may be utilized).

7.4.1.2. Pipette 7.5mL of *Sample Preparation* and 7.5mL of Purified Water into a glass-stoppered flask.

7.4.2. Formaldehyde Standard (5ppm CH_2O) Solution R:

7.4.2.1. Immediately before use, pipette the amount determined by the equation below of 16% (w/v) Formaldehyde Reference Standard into a volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix thoroughly.

$$\text{Formaldehyde Volume (mL)} = \frac{\big(Final\ Volume\ (mL)\big)\big(Final\ Concentration\ (ppm)\big)}{\big(Formaldehyde\ Purity\ (\%)\big)(10000)}$$

7.4.3. Standard Preparation:

7.4.3.1. Pipette 7.5mL of Formaldehyde Standard (5ppm CH_2O) Solution R and 7.5mL of Purified Water into a glass-stoppered flask.

7.4.4. Analysis:

7.4.4.1. To both the Sample and Standard, add 1mL of Decolorized Pararosaniline Solution R, stopper the flask, and allow to stand at 25°C ± 1°C for 1 hour.

7.4.4.2. After an hour, measure the absorbance of the standard and the sample at 552nm using the UV/Vis Spectrophotometer.

7.4.5. Acceptance Criteria:

7.4.5.1. The test is not valid unless the standard is pink.

7.4.5.2. The absorbance of the sample solution is not more than that of the standard solution to report as <10ppm.

7.5. **ALDEHYDES AND REDUCING SUBSTANCES (ChP):**

7.5.1. Sample Preparation:

7.5.1.1. Weigh 1.0 grams of sample into a 50mL volumetric flask, dissolve in 25mL of Purified Water, add 2mL of freshly prepared 10% Methylbenzo-2-Thiazolonehydrazone Hydrochloride Solution, adjust the pH to 4.0 with 0.02N Sodium Hydroxide, and allow to stand for 30 minutes.

7.5.1.2. Add 5mL of freshly prepared 0.5% Ferric Chloride Solution, mix well, and allow to stand for 5 minutes.

7.5.1.3. Dilute to volume with Methanol and mix well.

7.5.2. Formaldehyde Standard (5ppm CH_2O) Solution R:

7.5.2.1. Immediately before use, pipette the amount determined by the equation below of 16% (w/v) Formaldehyde Reference Standard into a volumetric flask, dissolve in Purified Water, fill to volume with Purified Water, and mix thoroughly.

$$\text{Formaldehyde Volume (mL)} = \frac{\big(Final\ Volume\ (mL)\big)\big(Final\ Concentration\ (ppm)\big)}{\big(Formaldehyde\ Purity\ (\%)\big)(10000)}$$

7.5.3. Standard Preparation:

7.5.3.1. Pipette 2mL of *Formaldehyde Standard (5ppm CH_2O) Solution R* into a 50mL volumetric flask, dissolve in 25mL of Purified Water, add 2mL of freshly prepared 10% Methylbenzo-2-Thiazolonehydrazone Hydrochloride Solution, adjust the pH to 4.0 with 0.02N Sodium Hydroxide, and allow to stand for 30 minutes.

7.5.3.2. Add 5mL of freshly prepared 0.5% Ferric Chloride Solution, mix well, and allow to stand for 5 minutes.

7.5.3.3. Dilute to volume with Methanol and mix well.

7.5.4. Analysis:

7.5.4.1. Measure the absorbance of the standard and the sample at 655nm using the UV/Vis Spectrophotometer.

7.5.4.2. The absorbance of the sample solution is not more than that of the standard solution to report as Passes Test.

7.6. **ACROLEIN, GLUCOSE, AND OTHER REDUCING SUBSTANCES (JP):**

7.6.1. To 1.0 gram of sample, add 1mL of Ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes, no yellow color is produced.

7.6.2. Take the solution out of the water bath, immediately add 3 drops of 0.1N Silver Nitrate and allow to stand in a dark place for 5 minutes. The color of the solution does not change and no turbidity is produced to report as Passes Test.

7.7. **AMMONIUM (JP):**

7.7.1. To 5mL of sample, add 5mL of 10% Sodium Hydroxide and bring to a boil.

7.7.2. The gas evolved does not change moistened red litmus paper to blue to report as Passes Test.

7.8. **AMMONIUM (ChP):**

7.8.1. Dissolve 4.0 grams of sample in 5mL of 10% Potassium Hydroxide solution and mix well.

7.8.2. Allow to stand at 60°C for 5 minutes.

7.8.3. No odor of Ammonia is produced to report as Passes Test.

7.9. **APPEARANCE (EP) (JP) (ChP):**

7.9.1. Refer to *Appearance of Solution (EP)* testing in Section 7.10 below.

7.10. **APPEARANCE OF SOLUTION (EP):**

7.10.1. Sample Preparation:

7.10.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well. (Solution S may be utilized).

7.10.2. Clarity:

7.10.2.1. Analyze the *Sample Preparation* for turbidity using a calibrated turbidimeter.

7.10.2.2. Acceptance Criteria:

7.10.2.2.1. The turbidity result may not exceed 3NTU to report as clear.

7.10.3. Color:

7.10.3.1. Dilute 10mL of *Sample Preparation* to 25mL with Purified Water and mix well.

7.10.3.2. In an area with sufficient lighting, compare the color of the *Dilute Sample Preparation* to Purified Water.

7.10.3.3. Acceptance Criteria:

7.10.3.3.1. The color of the *Dilute Sample Preparation* may not be more intense than the color of Purified Water to report as colorless.

7.10.4. To report as Passes Test, the solution must be both Clear and Colorless.

7.11. **ARSENIC (JP) (ChP):**

7.11.1. Refer to BSI-ATM-0116, Analytical Method for the Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin for sample preparation and analysis.

7.12. **ASSAY (ANHYDROUS BASIS) (USP) (EP) (JP) (ChP):**

7.12.1. **Sodium Periodate Solution**:

7.12.1.1. Dissolve 60 grams of Sodium Metaperiodate in 120mL of 0.1N Sulfuric Acid, dilute to 1000mL with Purified Water, and mix well.

7.12.1.1.1. Do not heat to dissolve the Sodium metaperiodate.

7.12.1.1.2. If the solution is not clear, pass through a sintered-glass filter.

7.12.1.1.3. Store the solution in a glass-stoppered, light resistant container.

7.12.2. **Sodium Periodate Solution Test Suitability**:

7.12.2.1. Dilute Sodium Periodate Solution:

7.12.2.1.1. Pipette 10mL of *Sodium Periodate Solution* into a 250mL volumetric flask, fill to volume with Purified Water, and mix well.

7.12.2.2. Blank:

7.12.2.2.1. Pipette 50mL of *Dilute Sodium Periodate Solution* into 50mL of Purified Water, and mix well.

7.12.2.3. Test Solution:

7.12.2.3.1. Dissolve 0.55 grams of Glycerin in 50mL of Purified Water, add 50mL of *Dilute Sodium Periodate Solution*, and mix well.

7.12.2.3.1.1. Note: If the beaker exceeds the maximum weight of the analytical balance, weigh the sample into a smaller beaker, dissolve in 50mL of Purified Water, and analytically transfer the sample to a suitable beaker.

7.12.2.4. Suitability Analysis:

7.12.2.4.1. Allow the *Blank* and *Test Solution* to stand for 30 minutes.

7.12.2.4.2. After 30 minutes, to each add 5mL of concentrated Hydrochloric Acid, 10mL of Potassium Iodide TS, and mix well.

7.12.2.4.3. Allow to stand for 5 minutes, add 100mL of Purified Water, and titrate with 0.1N Sodium Thiosulfate.

7.12.2.4.4. As the endpoint is approached, add 3mL of Starch TS and titrate to a colorless endpoint.

7.12.2.5. Acceptance Criteria:

7.12.2.5.1. The ratio of the volume of 0.1N Sodium Thiosulfate required for the *Test Solution* to that required for the *Blank Solution* should be between 0.750 and 0.765.

7.12.3. **Glycerin Assay**:

7.12.3.1. Calibrate the pH Probe as per Standardization of Titrants.

7.12.3.2. Standardize or Daily Check 0.1N Sodium Hydroxide as per Standardization of Titrants.

7.12.3.3. Blank:

7.12.3.3.1. **Note:** Perform the Blank determination within 24 hours of analysis or after a reagent change.

7.12.3.3.2. Add 50mL of Purified Water to a suitable beaker, add Bromothymol Blue TS, and acidify with 0.2N Sulfuric Acid to a definite green or greenish-yellow color, if necessary.

7.12.3.3.3. Neutralize with 0.05N Sodium Hydroxide to a definite blue endpoint, free from green color.

7.12.3.4. Glycerin Samples:

7.12.3.4.1. Dissolve 0.30 grams of Glycerin sample in 50mL of Purified Water in a suitable beaker.

7.12.3.4.1.1. Note: If the beaker exceeds the maximum weight of the analytical balance, weigh the sample into a smaller beaker, dissolve in 50mL of Purified Water, and analytically transfer the sample to a suitable beaker.

7.12.3.4.2. Add Bromothymol Blue TS and acidify with 0.2N Sulfuric Acid to a definite green or greenish-yellow color.

7.12.3.4.3. Neutralize with 0.05N Sodium Hydroxide to a definite blue endpoint, free from green color.

7.12.3.5. Analysis:

7.12.3.5.1. To the *Blank* and *Glycerin Samples*, add 50mL of *Sodium Periodate Solution*, mix by swirling, cover with a watch glass, and allow to stand at room temperature (not exceeding 35°C) in the dark for 30 minutes.

7.12.3.5.2. After 30 minutes, add 10mL of a mixture of equal volumes of Ethylene Glycol and Purified Water, and allow to stand for 20 minutes.

7.12.3.5.3. Dilute to 300mL with Purified Water and titrate with 0.1N Sodium Hydroxide to a pH of 8.1 ± 0.1 for the Glycerin Samples and 6.5 ± 0.1 for the Blank using the Metrohm Titrando 907.

7.12.3.5.4. Each mL of 0.1N Sodium Hydroxide, after correction for the blank, is equivalent to 9.210mg of Glycerin.

7.12.3.5.5. Calculate the %Glycerin (As-Is) using the following equation in the Metrohm® Tiamo™ software:

$$\%\text{Glycerin (as-is)} = \frac{(\text{Sample Endpoint (mL)} - \text{Blank Endpoint (mL)})\ (\text{Titer Value (N)})\ (9.210)}{Sample\ Weight\ (g)}$$

7.12.3.5.6. Calculate the %Glycerin (Anhydrous Basis) using the following equation:

$$\%\text{Glycerin (Anhydrous Basis)} = \frac{\%\text{Glycerin (as-is)} \times 100}{100 - \text{Karl Fischer Value (\%)}}$$

7.13. **BACTERIAL ENDOTOXINS (ChP) :**

7.13.1. Accurately weigh between 0.10 grams and 0.20 grams of sample into a sterile tube.

7.13.2. Dilute to 10mL with LAL Reagent Water, dissolve, and mix thoroughly for a final concentration of between 0.010g/mL and 0.020g/mL.

7.13.3. Refer to BSI-SOP-0345, Endosafe NexGen PTS Endotoxin Reader SOP for instrument operation and sample analysis.

7.14. **CALCIUM (JP) (ChP) :**

7.14.1. Refer to BSI-ATM-0116, Analytical Method for the Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin for sample preparation and analysis.

7.15. **CHLORIDES (ChP) :**

7.15.1. Sample Preparation:

7.15.1.1. Dissolve 5.0 grams of sample into 25mL of Purified Water.

7.15.1.1.1. **Note:** if the solution is alkaline, neutralize with nitric acid dropwise.

7.15.1.2. Add 10 mL of Dilute Nitric Acid and filter if necessary.

7.15.1.3. Transfer the solution to a 50mL Nessler Color Comparison Tube.

7.15.1.4. Add Purified Water to produce 40mL, and mix well.

7.15.2. Standard Preparation:

7.15.2.1. Transfer 3.0mL of Sodium Chloride Standard Solution to a 50mL Nessler Tube.

7.15.2.2. Add 10mL of Dilute Nitric Acid.

7.15.2.3. Add Purified water to produce 40mL, and mix well.

7.15.3. Analysis:

7.15.3.1. To each of the solutions prepared above, add 1.0mL of 0.1N Silver Nitrate.

7.15.3.2. Dilute with Purified Water to 50mL and mix well.

7.15.3.3. Allow the solutions to stand in the dark for 5 minutes.

7.15.3.4. After 5 minutes, examine the tubes laterally against a dark background.

7.15.3.5. Any opalescence in the *Sample Preparation* must not exceed that of the *Standard Preparation* in order to report as ≤ 6ppm.

7.16. **CHLORIDES (EP) (JP) :**

7.16.1. Sample Preparation:

7.16.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well (Solution S may be utilized).

7.16.1.2. Dilute 1mL of *Sample Preparation* to 15mL with Purified Water.

7.16.2. Standard Preparation:

7.16.2.1. Chloride Standard Solution (5ppm Cl^-) R: Immediately before use, dilute 1mL of *Chloride Stock Solution (500ppm Cl^-)* to 100mL with Purified Water and mix well.

7.16.2.2. Dilute 1mL of *Chloride Standard Solution (5ppm Cl) R* to 15mL with Purified Water.

7.16.3. Analysis:

7.16.3.1. To the Sample and Standard Preparations, add 1mL of Dilute Nitric Acid R, 1mL of 0.1N Silver Nitrate, and mix well.

7.16.3.2. Allow to stand protected from light for 5 minutes.
7.16.3.3. After 5 minutes, examine the tubes laterally against a dark background.
7.16.3.4. Any opalescence in the Sample Preparation must not exceed that in the standard to report as ≤ 10ppm.

7.17. **CHLORIDES (USP):**

7.17.1. Sample Preparation:
7.17.1.1. Weigh 7.0 grams of sample into a 50mL Nessler Color Comparison Tube and dissolve in approximately 40mL of Purified Water. If necessary, neutralize the solution with Nitric Acid to litmus.
7.17.2. Standard Preparation:
7.17.2.1. Pipette 0.10mL of 0.02N Hydrochloric Acid into approximately 40mL of Purified Water in a 50mL Nessler Color Comparison Tube.
7.17.3. Analysis:
7.17.3.1. To both the samples and standards, added 1mL of concentrated Nitric Acid, 1mL of 0.1N Silver Nitrate, fill to volume with Purified Water, cover with parafilm and mix by inversion.
7.17.3.2. Allow to stand for 5 minutes utilizing a calibrated timer then viewed tubes against a dark background.
7.17.3.3. The turbidity of the sample preparation does not exceed that produced by the standard preparation to report as ≤ 10ppm.

7.18. **COLOR (ChP):**

7.18.1. Standard Preparation:
7.18.1.1. Pipette 0.2mL of Potassium Dichromate CS into a 50mL Nessler Color Comparison Tube, dilute to volume with Purified Water, and mix well.
7.18.2. Sample Preparation:
7.18.2.1. Fill a 50mL Nessler Color Comparison Tube to volume with Glycerin sample.
7.18.3. Analysis:
7.18.3.1. View the Standard Preparation and Sample Preparation downward against a white surface.
7.18.3.2. The color in the Sample Preparation is not more intense than the color in the Standard Preparation to report as “Passes Test.”

7.19. **COLOR (JP) (USP):**

7.19.1. Standard Preparation:
7.19.1.1. Pipette 0.40mL of Ferric Chloride CS into a 50mL Nessler Color Comparison Tube, dilute to volume with Purified Water, and mix well.
7.19.2. Sample Preparation:
7.19.2.1. Fill a 50mL Nessler Color Comparison Tube to volume with Glycerin sample.
7.19.3. Analysis:
7.19.3.1. View the standard Preparation and Sample Preparation downward against a white surface.
7.19.3.2. The color in the Sample Preparation is not darker than the color in the Standard Preparation to report as Passes Test.”

7.20. **DIHYDROXYACETONE:**

7.20.1. Refer to BSI-ATM-0135, Limit of Specified Impurities in Glycerin by HPLC, for sample preparation and analysis.

7.21. **ELEMENTAL IMPURITIES:**

7.21.1. Refer to BSI-ATM-0116, Analytical Method for the Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin for sample preparation and analysis.

7.22. **ESTERS (EP):**

7.22.1. To the final solution obtained from the Acidity or Alkalinity analysis in Section 7.2, add 10mL of 0.1N Sodium Hydroxide.

7.22.2. Boil under a reflux condenser for 5 minutes and allow to cool.

7.22.3. Add 0.5mL of Phenolphthalein Solution R and titrate with 0.1N Hydrochloric Acid.

7.22.4. No less than 8.0mL of 0.1N Hydrochloric Acid is required to change the color of the indicator to report as passes test.

7.23. **FATTY ACIDS AND ESTERS (ChP):**

7.23.1. Blank Preparation:

7.23.1.1. To 40mL of freshly boiled and cooled Purified Water, accurately add 10mL of 0.1N Sodium Hydroxide and mix thoroughly.

7.23.1.2. Boil the mixture for 5 minutes, allow to cool, and add a few drops of *Phenolphthalein TS*.

7.23.2. Sample Solution:

7.23.2.1. Dissolve 40.0 grams of Glycerin sample in 40mL of freshly boiled and cooled Purified Water, accurately add 10mL of 0.1N Sodium Hydroxide, and mix thoroughly.

7.23.2.2. Boil the mixture for 5 minutes, allow to cool, and add a few drops of *Phenolphthalein TS*.

7.23.3. Analysis:

7.23.3.1. To both the Blank Preparation and Sample Preparation, titrate with 0.1N Hydrochloric Acid to a colorless endpoint.

7.23.3.2. Calculate the amount of 0.1N Sodium Hydroxide consumed using the following equation:

$$0.1N\ NaOH\ Consumed = 10.0\ mL - mL\ of\ titrant\ added\ (0.1N\ HCl)$$

7.23.3.3. Not more than 2.0mL of 0.1N Sodium Hydroxide is consumed, when corrected for the Blank Preparation, to report as “Passes Test”.

7.24. **FATTY ACIDS AND ESTERS (JP):**

7.24.1. Blank Preparation:

7.24.1.1. To 50mL of freshly boiled and cooled Purified Water, accurately add 10mL of 0.1N Sodium Hydroxide, and mix thoroughly.

7.24.1.2. Boil the mixture for 15 minutes, allow to cool, and add 3 drops of Phenolphthalein TS.

7.24.2. Sample Solution:

7.24.2.1. Dissolve 50 grams of Glycerin sample in 50mL of freshly boiled and cooled Purified Water, accurately add 10mL of 0.1N Sodium Hydroxide, and mix thoroughly.

7.24.2.2. Boil the mixture for 15 minutes, allow to cool, and add 3 drops of Phenolphthalein TS.

7.24.3. Analysis:

7.24.3.1. To both the Blank Preparation and Sample Preparation, titrate with 0.1N Hydrochloric Acid to a colorless endpoint.

7.24.3.2. Calculate the amount of 0.1N Sodium Hydroxide consumed using the following equation:

$$0.1N\ NaOH\ Consumed = 10.0\ mL - mL\ of\ titrant\ added\ (0.1N\ HCl)$$

7.24.3.3. Not more than 3.0mL of 0.1N Sodium Hydroxide is consumed, when corrected for the Blank Preparation, to report as "Passes Test".

7.25. **FATTY ACIDS AND ESTERS (USP):**

7.25.1. Blank Preparation:

7.25.1.1. To 50mL of freshly boiled Purified Water, add 5mL of 0.5N Sodium Hydroxide.

7.25.1.2. Boil the mixture for 5 minutes, cool, and add Phenolphthalein TS.

7.25.2. Sample Preparation:

7.25.2.1. Mix 50 grams of Glycerin sample with 50mL of freshly boiled Purified Water and add 5mL of 0.5N Sodium Hydroxide.

7.25.2.2. Boil the mixture for 5 minutes, cool, and add Phenolphthalein TS.

7.25.3. Analysis:

7.25.3.1. To both the Blank Preparation and the Sample Preparation, titrate the excess Sodium Hydroxide with 0.5N Hydrochloric Acid.

7.25.3.2. Calculate the amount of 0.5N Sodium Hydroxide consumed using the following equation:

$$0.5N\ NaOH\ Consumed = 10.0\ mL - mL\ of\ titrant\ added\ (0.5N\ HCl)$$

7.25.3.3. No more than 1mL of 0.5N Sodium Hydroxide is consumed to report as Passes Test.

7.26. **FORMALDEHYDE:**

7.26.1. Refer to BSI-ATM-0140, Analytical Method: Quantification of Formaldehyde by GC-MS (Excipient) for sample preparation and analysis.

7.27. **GLYCERALDEHYDE:**

7.27.1. Refer to BSI-ATM-0135, Limit of Specified Impurities in Glycerin by HPLC, for sample preparation and analysis.

7.28. **HALOGENATED COMPOUNDS (ChP):**

7.28.1. Sample Preparation:

7.28.1.1. Dissolve 5.0 grams of Glycerin sample in 10mL of Purified Water, add 1mL of 2N Sodium Hydroxide, add 50mg of Halogen-Free Nickel-Aluminum Alloy, and mix thoroughly.

7.28.1.2. Heat in a water bath for 10 minutes, allow to cool to room temperature, and filter into a 50mL Nessler Color Comparison Tube.

7.28.1.3. Wash the flask and the residue with 20mL of Purified Water in portions and add to the 50mL Nessler Color Comparison Tube.

7.28.1.4. Add 0.5mL of concentrated Nitric Acid and 0.5mL of 0.1N Silver Nitrate, fill to volume with Purified Water, and mix well.

7.28.2. Standard Preparation:

7.28.2.1. Pipette 15mL of *Sodium Chloride Standard Solution* into 10mL of Purified Water, add 1mL of 2N Sodium Hydroxide, add 50mg of Halogen-Free Nickel-Aluminum Alloy, and mix thoroughly.

7.28.2.2. Heat in a water bath for 10 minutes, allow to cool to room temperature, and filter into a 50mL Nessler Color Comparison Tube.

7.28.2.3. Wash the flask and the residue with 20mL of Purified Water in portions and add to the 50mL Nessler Color Comparison Tube.

7.28.2.4. Add 0.5mL of concentrated Nitric Acid and 0.5mL of 0.1N Silver Nitrate, fill to volume with Purified Water, and mix well.

7.28.3. Analysis:

7.28.3.1. Any opalescence in the sample preparation is not more intense than that in the standard preparation to report as ≤ 30ppm.

7.29. **HALOGENATED COMPOUNDS (EP):**

7.29.1. Sample Preparation:

7.29.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well (Solution S may be utilized).

7.29.1.2. To 10mL of *Sample Preparation*, add 1mL of Dilute Sodium Hydroxide Solution R, 5mL of Purified Water, 50mg of Halogen-Free Nickel-Aluminum Alloy R, and mix thoroughly.

7.29.1.3. Heat in a water bath for 10 minutes, allow to cool, and filter. Rinse the flask and the filter with Purified Water until 25mL of filtrate is obtained.

7.29.1.4. To 5mL of the filtrate add 4mL of Reagent Alcohol, 2.5mL of Purified Water, 0.5mL of concentrated Nitric Acid, 0.05mL of Silver Nitrate Solution R2, and mix thoroughly.

7.29.2. Standard Preparation:

7.29.2.1. Mix 7.0mL of Chloride Standard Solution (5ppm Cl) R, 4mL of Reagent Alcohol, 0.5mL of Purified Water, 0.5mL of concentrated Nitric Acid, 0.05mL of Silver Nitrate Solution R2, and mix thoroughly.

7.29.3. Analysis:

7.29.3.1. Allow both solutions to stand for 2 minutes.

7.29.3.2. Any opalescence in the sample preparation is not more intense than that in the standard preparation to report as ≤ 35ppm.

7.30. **HEAVY METALS (ChP) (JP):**

7.30.1. Refer to BSI-ATM-0116, Analytical Method for the Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin for sample preparation and analysis.

7.31. **HYDROXYACETONE:**

7.31.1. Refer to BSI-ATM-0135, Limit of Specified Impurities in Glycerin by HPLC, for sample preparation and analysis.

7.32. **IDENTIFICATION, IR (ChP) (EP-B) (JP) (USP-A):**

7.32.1. Follow the Spectrum Two UATR SOP.

7.32.2. ChP, JP, USP-A Sample Preparation: Analyze sample as-is.

7.32.3. EP-B Sample Preparation: Thoroughly mix 5mL of sample with 1mL of Purified Water.

7.33. **LIMIT OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL (USP-B):**

7.33.1. Refer to BSI-ATM-0134, Limit of Related Substances in Glycerin via GC-FID for sample preparation and analysis.

7.34. **IDENTIFICATION C (USP):**

7.34.1. Refer to BSI-ATM-0134, Limit of Related Substances in Glycerin via GC-FID for sample preparation and analysis.

7.34.2. Acceptance Criteria:

7.34.2.1. The retention time of the Glycerin peak of the Sample Solution corresponds to that obtained in the Standard Solution.

7.35. **IRON (ChP):**

7.35.1. Refer to BSI-ATM-0116, Analytical Method for the Determination of Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Glycerin for sample preparation and analysis.

7.36. **LIMIT OF CHLORINATED COMPOUNDS (USP):**

7.36.1. Sample Preparation:

7.36.1.1. Transfer 5 grams of sample into a dry 100mL round-bottom flask, add 15mL of Morpholine, and connect the flask by a ground joint to a reflux condenser.

7.36.1.2. Reflux gently for 3 hours.

7.36.1.3. After 3 hours, rinse the condenser with 10mL of Purified Water into the round-bottom flask.

7.36.1.4. Cautiously acidify with concentrated Nitric Acid and transfer the solution to a 50mL Nessler Color Comparison Tube.

7.36.2. Standard Preparation:

7.36.2.1. Pipette 0.20mL of 0.02N Hydrochloric Acid into a dry 100mL round-bottom flask and add 15mL of Morpholine.

7.36.2.2. Add 10mL of Purified Water, cautiously acidify with concentrated Nitric Acid, and transfer to a 50mL Nessler Color Comparison Tube.

7.36.3. Analysis:

7.36.3.1. To the Sample and the Standard, add 0.50mL of 0.1N Silver Nitrate, dilute to volume with Purified Water, and mix well.

7.36.3.2. The turbidity of the Sample is not greater than that of the Standard to report as ≤ 30ppm Chloride.

7.37. **MICROBIAL CONTENT:**

7.37.1. Microbial analysis will be performed by an outside testing laboratory.

7.37.2. Package and send NLT 35 grams of sample to an approved contract laboratory.

7.37.3. Analyses:

7.37.3.1. Total Aerobic Microbial Count (TAMC)

7.37.3.2. Total Yeast Microbial Count (TYMC)

7.37.3.3. Absence of Escherichia Coli

7.38. **READILY CARBONIZABLE SUBSTANCES (ChP):**

7.38.1. Sample Preparation:

7.38.1.1. Weigh 8.0 grams of sample into a suitable vessel and add 5mL of concentrated Sulfuric Acid dropwise while shaking, ensuring the temperature does not exceed 20°C, and allow to sit for 1 hour.

7.38.2. Standard Preparation:

7.38.2.1. In a suitable vessel, pipette 0.2mL of Cobaltous Chloride CS, 1.6mL of Potassium Dichromate CS, 8.2mL of Purified Water, and mix thoroughly.

7.38.3. Analysis:

7.38.3.1. Any color produced by the Sample Preparation is not more intense than that produced by the Standard Preparation to report as “Passes Test”.

7.39. **READILY CARBONIZABLE SUBSTANCES (JP):**

7.39.1. Pipette 5mL of sample into a suitable vessel and add 5mL of Sulfuric Acid for Readily Carbonizable Substances, gently mixing at a temperature between 18°C and 20°C.

7.39.2. Allow to stand for 1 hour between 15°C and 25°C.

7.39.3. The solution should have no more color than Matching Fluid H to report as “Passes Test”.

7.40. **REFRACTIVE INDEX @ 20°C (ChP) (EP-A) (JP):**

7.40.1. Refer to BSI-SOP-0342, FisherBrand™ Handheld Digital Brix/RI Refractometer, for sample preparation and analysis.

7.40.2. Measure the refractive index at 20°C ± 0.5°C.

7.41. **RELATED SUBSTANCES:**

7.41.1. Refer to BSI-ATM-0134, Limit of Related Substances in Glycerin via GC-FID, for sample preparation and analysis.

7.42. **RESIDUAL SOLVENTS - METHANOL:**

7.42.1. Refer to BSI-ATM-0136, Residual Solvents by Headspace GC-FID: Glycerin, for sample preparation and analysis.

7.43. **RESIDUE ON IGNITION (ChP) (JP) (USP); SULFATED ASH (EP):**

7.43.1. Turn on the Muffle Furnace and allow it to stabilize at 600°C. Follow the Muffle Furnace calibration procedure for operation of the furnace.

7.43.2. Inspect a quartz crucible for cracks, chips, and discoloration.

7.43.3. Utilize forceps to insert and remove the quartz crucible from the furnace.

7.43.4. Ignite a quartz crucible at 600° ± 50°C for 30 minutes. Cool in a desiccator for 1.5 hours, and weigh using an analytical balance.

7.43.5. Weigh 5.0 grams of sample into the previously ignited quartz crucible, heat the dish until the sample ignites and allow it to burn without further application of heat in a draft free area.

7.43.6. Allow the sample to come to room temperature and moisten with 0.5mL of concentrated Sulfuric Acid.

7.43.7. Volatilize the sample until the sample is thoroughly charred and white fumes are no longer evolved. Heat the sample slowly, so that the sample does not boil over and sample is not lost.

7.43.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.43.7.2. Continue to heat the sample until all excess sulfuric acid has been volatilized.

7.43.8. Ignite in the muffle furnace at 600°C ± 50°C for 15 minutes or until all carbon has been removed.

7.43.9. Cool in a desiccator for 1.5 hours and weigh on an analytical balance.

7.43.10. Calculate the %ROI as follows:

$$\%ROI = \frac{Residue\ Weight\ (g)}{Sample\ Weight\ (g)} \times 100$$

7.43.11. If the amount of residue exceeds the limit specified, repeat the moistening with concentrated sulfuric acid using up to 1mL, heat to char, ignite at 600°C ± 50°C, cool in a desiccator, and reweigh until two consecutive weighings of the residue do not differ by more than 0.0005 grams.

7.44. **SPECIFIC GRAVITY (JP); RELATIVE DENSITY (ChP) (EP-C) @ 20°C:**

7.44.1. Refer to BSI-SOP-0308, Pycnometer SOP, for sample preparation and analysis.

7.44.2. Measure the Specific Gravity / Relative Density at 20°C.

7.45. **SPECIFIC GRAVITY @ 25°C (USP):**

7.45.1. Refer to BSI-SOP-0308, Pycnometer SOP, for sample preparation and analysis.

7.45.2. Measure the Specific Gravity at 25°C.

7.46. **SUGARS (ChP) (EP):**

7.46.1. Sample Preparation:

7.46.1.1. Weigh 100 grams of Glycerin sample into a 200mL volumetric flask, dissolve with Purified Water, fill to volume with Purified Water, and mix well (Solution S may be utilized).

7.46.2. Analysis:

7.46.2.1. To 10mL of *Sample Preparation*, add 1mL of Dilute Sulfuric Acid R and heat in a water bath for 5 minutes.

7.46.2.2. Add 3mL of 85mg/mL Sodium Hydroxide, mix, and add dropwise 1mL of freshly prepared Copper Sulfate Solution R and mix well. The solution is clear and blue.

7.46.2.2.1. **Note:** Precipitate will form after adding the Copper Sulfate Solution R, but it should dissolve readily with agitation.

7.46.2.3. Continue heating on the water bath for 5 minutes. The solution remains blue and no precipitate is formed to report as "Passes Test."

7.47. **SULFATES (ChP):**

7.47.1. Sample Preparation:

7.47.1.1. Weigh 10 grams of sample into a 50mL Nessler Color Comparison Tube and dissolve in ~40mL of Purified Water. If necessary, neutralize the solution with Hydrochloric Acid to litmus and filter.

7.47.2. Standard Preparation:

7.47.2.1. Pipette 2.0mL of Potassium Sulfate Standard Solution into a 50mL Nessler Color Comparison Tube, dilute to 40mL with Purified Water, and add 2mL of Dilute Hydrochloric Acid.

7.47.3. Analysis:

7.47.3.1. To the sample and the standard, add 5mL of 25% Barium Chloride, dilute to 50mL with Purified Water, cover with parafilm, and mix by inversion.

7.47.3.2. Allow to sit for 10 minutes.

7.47.3.3. Any opalescence produced in the Sample Preparation should not exceed that produced by the Standard Preparation when viewed from above against a black background to report as ≤ 20ppm.

7.48. **SULFATES (JP):**

7.48.1. Sample Preparation:

7.48.1.1. Weigh 10.0 grams of sample into a 50mL Nessler Color Comparison Tube, dissolve and dilute to 40mL with Purified Water, add 1mL of Dilute Hydrochloric Acid, dilute to 50mL with Purified Water, and mix well.

7.48.2. Standard Preparation:

7.48.2.1. Pipette 0.40mL of 0.01N Sulfuric Acid into a 50mL Nessler Color Comparison Tube, add 1mL of Dilute Hydrochloric Acid, dilute to 50mL with Purified water, and mix well.

7.48.3. Analysis:

7.48.3.1. To the Sample and Standard, add 2mL of Barium Chloride TS, cover with parafilm, mix by inversion, and allow to sit for 10 minutes.

7.48.3.2. Any turbidity produced in the Sample Preparation should not exceed that produced in the Standard Preparation when viewed from above against a black background to report as ≤ 20ppm.

7.49. **SULFATES (USP):**

7.49.1. Sample Preparation:

7.49.1.1. Weigh 10 grams of sample into a 50mL Nessler Color Comparison Tube. Dissolve in ~40mL of Purified Water. If necessary, neutralize the solution with Hydrochloric Acid to litmus.

7.49.2. Standard Preparation:

7.49.2.1. Prepare a standard solution by pipetting 0.20mL of 0.02N Sulfuric Acid into a 50mL Nessler Color Comparison Tube and dilute to ~40mL with Purified Water.

7.49.3. Procedure:

7.49.3.1. To the Sample and the Standard, add 1mL of 3N Hydrochloric Acid, 3mL of Barium Chloride TS, and dilute to 50mL with Purified Water.

7.49.3.2. Cover with parafilm, mix by inversion, and allow to sit for 10 minutes.

7.49.3.3. Any turbidity produced in the Sample Preparation should not exceed that produced by the Standard Preparation when viewed from above against a black background to report as ≤ 20ppm.

7.50. **WATER CONTENT (KARL FISCHER TITRATION):**

7.50.1. Standardize Karl Fischer titrant (Composite 5) as per the Standardization of Titrants SOP

7.50.2. Fill a 1-mL, disposable syringe with glycerin sample by drawing up the sample slowly, ensuring not to capture any air bubbles.

7.50.3. Place the filled syringe in a weigh boat and place onto the analytical balance.

7.50.4. Tare the analytical balance.

7.50.5. Once the Karl Fischer vessel has conditioned, the Tiamo will report "Conditioning-OK." Press the green play button on Tiamo and a message will appear stating to add sample.

7.50.6. Transfer ~0.4mL of sample to the Karl Fischer vessel by removing the rubber septum and adding the sample into the titration vessel.

7.50.6.1. Do not leave the rubber septum open for longer than 20 seconds as this will allow moisture to enter the titration vessel.

7.50.6.2. Ensure no sample is lost on the septum walls.

7.50.6.3. **Note:** Sample volume is based off a 0.5-gram sample weight with an accepted glycerin density of 1.26g/mL.

7.50.7. Reweigh the sample syringe and enter the sample weight (difference of starting weight and syringe weight) when prompted.

7.50.8. The moisture content will then be determined by the Karl Fischer titration using the Metrohm Titrando 907.

$$\%Water = \frac{(mL\ of\ Composite\ 5)\left(\frac{mg}{mL}of\ Composite\ 5\right)(0.1)}{Sample\ Weight\ (g)}$$

8. COMPENDIAL METHOD REFERENCES:

8.1. Harmonized Compendial Analytical Methods

Table 1: Harmonized Compendial Analytical Methods
Appearance (EP) (JP) (ChP)
Acidity or Alkalinity (EP) (ChP)
Chlorides (EP) (JP)
Color (JP) (USP)
Identification, IR (ChP) (JP) (USP-A)
Refractive Index @ 20°C (ChP) (EP-A) (JP)
Residue On Ignition (ChP) (JP) (USP) / Sulfated Ash (EP)
Specific Gravity (JP) / Relative Density (ChP) (EP-C) @ 20°C
Sugars (ChP) (EP)

8.2. Validated Methods in Accordance with Compendial Monograph or General Chapters

Table 2: Validated Methods in Accordance with Compendial Monograph or General Chapters
Arsenic (JP) (ChP)
Assay (Anhydrous Basis) (USP) (EP) (JP) (ChP)
Appearance (EP) (JP) (ChP)
Bacterial Endotoxins (ChP)
Calcium (JP) (ChP)
Elemental Impurities
Heavy Metals (ChP) (JP)
Identification C (USP)
Iron (ChP)
Limit of Diethylene Glycol and Ethylene Glycol (USP-B)
Related Substances
Water Content (Karl Fischer Titration)

8.3. Non-Compendial Methods

Table 3: Non-Compendial Methods
Dihydroxyacetone
Formaldehyde
Glyceraldehyde
Hydroxyacetone
Residual Solvents - Methanol